

ABSTRACT

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ON THE MECHANISM OF COENZYME VITAMIN B₁₂ CATALYZED CONVERSION OF
METHYLMALONYL COENZYME A TO SUCCINYL COENZYME A: SYNTHESIS OF
MODEL SYSTEMS

Advisor: Professor Thomas W. Cole, Jr.

Thesis dated May 1980

2-Cyclopropyl succinic acid has been prepared from cyclopropyl carbinol.

This compound was designed as a model system for the mechanistic study of the reversible isomerization of methylmalonyl CoA to succinyl CoA.

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OF METHYLMALONYL COENZYME A TO SUCCINYL COENZYME A:
SYNTHESIS OF MODEL SYSTEMS

A THESIS
SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY
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INTRODUCTION

Pernicious anemia is a disorder evidenced by the excretion of large amounts of methylmalonic and propionic acids in the urine of those afflicted. The gastric juices of persons with the disease contain no hydrochloric acid and little or no pepsin. It is the defect in the production of mucoprotein (intrinsic factor) which gives rise to the condition.¹

In 1926 Minot and Murphy² found that pernicious anemia could be cured by administering large quantities of liver to their patients. The component in liver responsible for the cure is Vitamin B₁₂ which was isolated in 1948 in England by Smith³ and independently in the United States by Rickes and Folkers³ in the form of cyanocobalamin. Cobalamin is another name for the vitamin. Vitamin B₁₂ has been shown to be essential for normal red blood cell formation and maintenance of neural growth. It also serves as a coenzyme in some metabolic processes which will be mentioned later.⁴ The purpose of the mucoprotein is to incorporate the dietary vitamin.¹

The structure of vitamin B₁₂ (Fig. 1) was elucidated by a combination of chemical and X-ray diffraction methods. The large porphyrin-like component is a corrin ring system of which four are directly linked and six coordinate. A ribonucleoside is the major component. Unlike most ribonucleotides, an α -N-glycosyl linkage joins the base (5,6-dimethylbenzimidazole) and ribose-6-phosphate.

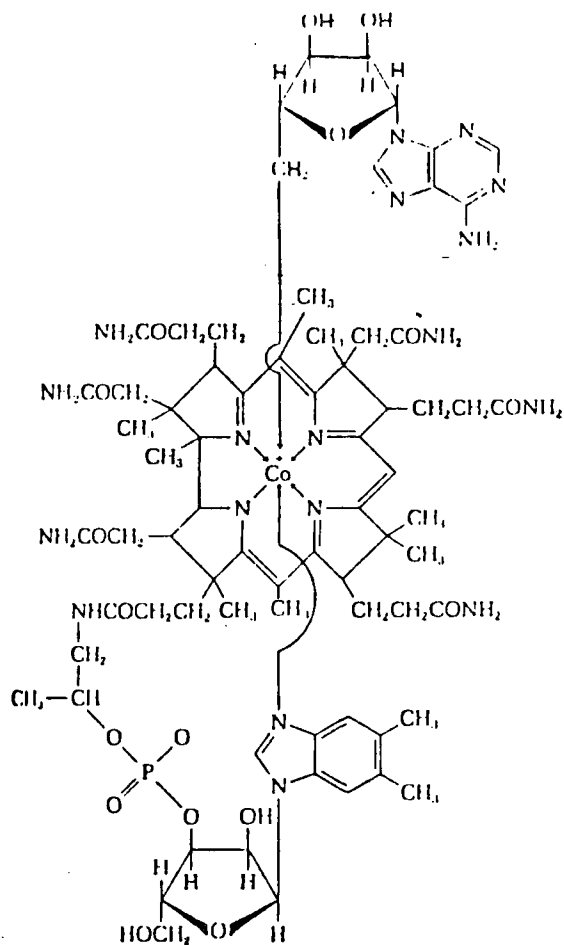
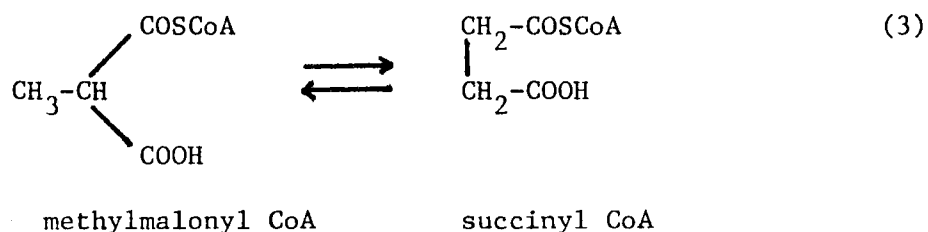
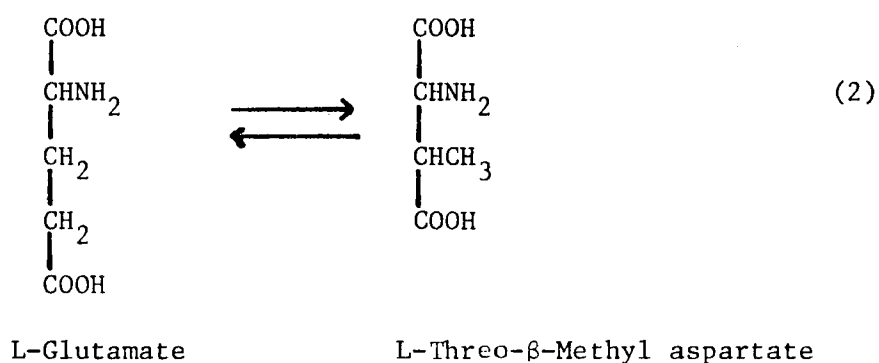
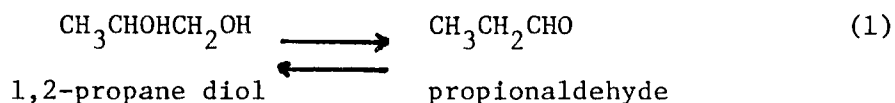


Fig. 1. Coenzyme Vitamin B₁₂ (deoxyadenosine cobalamin).*

*Taken from A. L. Lehninger, "Biochemistry," 2nd ed., Worth Publishers, Inc., New York, N. Y., 1975, p. 349.

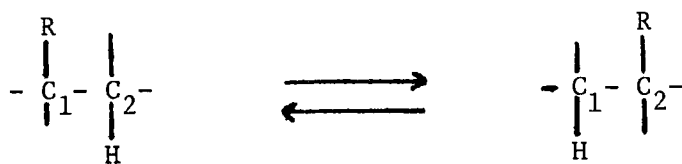
Coenzyme B₁₂ is a cofactor in ten known enzyme catalyzed rearrangements (Table 1), three of which are carbon skeleton rearrangement reactions as outlined in reactions 1-3 below.^{5,6}



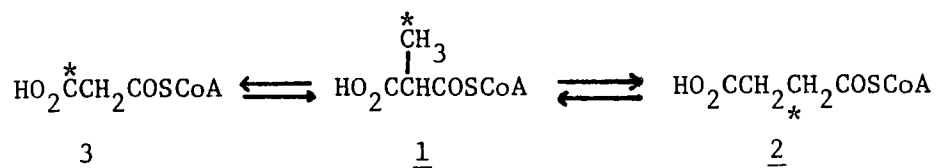
The outstanding feature of reactions 1-3 is the reversible conversion of an apparently unactivated methyl group to a methylene group followed by the incorporation of the latter into the backbone of the product. These features can be generalized by the following equation demonstrating the 1,2 shift of the hydrogen atom and an R group in opposite directions.⁵

Table 1. Coenzyme B₁₂-Dependent Rearrangements.

Reaction	Enzyme
L-Glutamate \rightleftharpoons L-Threo β -Methyl aspartate	Glutamate
L-Methylmalonyl-CoA \rightleftharpoons Succinyl-CoA	Methylmalonyl CoA mutase
2-Methyleneglutarate \rightleftharpoons Methylitaconate	2-Methyleneglutarate mutase
1,2-Propanediol \rightleftharpoons Propionaldehyde	Diol dehydrase
Ethylene glycol \rightleftharpoons Acetaldehyde	
Glycerol \rightleftharpoons β -Hydroxypropionaldehyde	Glycerol dehydrase
Ethanolamine \rightleftharpoons Acetaldehyde + NH ₄ ⁺	Ethanolamine ammonia lyase
2-Aminopropanol \rightleftharpoons Propionaldehyde + NH ₄ ⁺	
L- β -Lysine (L-3,6-diaminohexanoic acid) \rightleftharpoons 3,5-Diaminohexanoic acid	β -Lysine mutase
D-Lysine \rightleftharpoons 2,5-Diaminohexanoic acid	α -Lysine mutase
D-Ornithine \rightleftharpoons 2,4-Diaminopentanoic acid	Ornithine mutase
Ribose \rightleftharpoons Deoxyribose	Ribonucleotide reductase

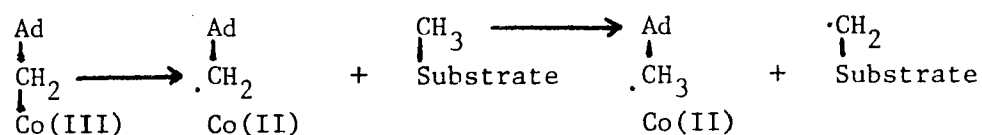


In reaction 1, the R group is -OH; reaction 2, -CHNH₂CO₂H; and in reaction 3, -COSCoA.⁵ In the reversible isomerization of methylmalonyl CoA to succinyl CoA, isotopic labelling studies (shown below) revealed -COSCoA as the migrating group. Had the migrating group been the carboxyl group, the labelled carbon would have been located in the position indicated with the asterisk in 3:⁷



The stereochemistry of the hydrogen is known in all three cases. Inversion of configuration is observed at carbon (labelled with asterisk in structures 1 and 2) to which hydrogen migrates in the propanediol dehydration reaction and in the glutamate isomerization reaction. In contrast, retention of configuration is observed at the corresponding carbon in the methylmalonyl CoA isomerization. The hydrogen atom occupies the same steric position as was previously occupied by the acetyl CoA group. Hydrogen migration is intermolecular and is mediated by the deoxyadenosine of the coenzyme (Scheme I).

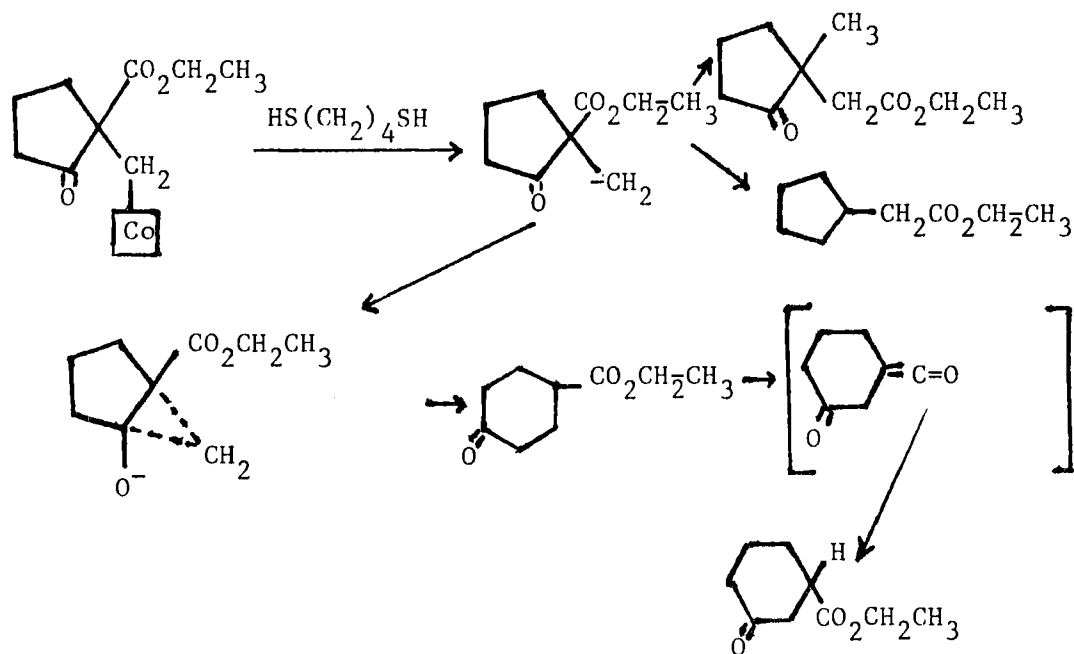
Scheme I



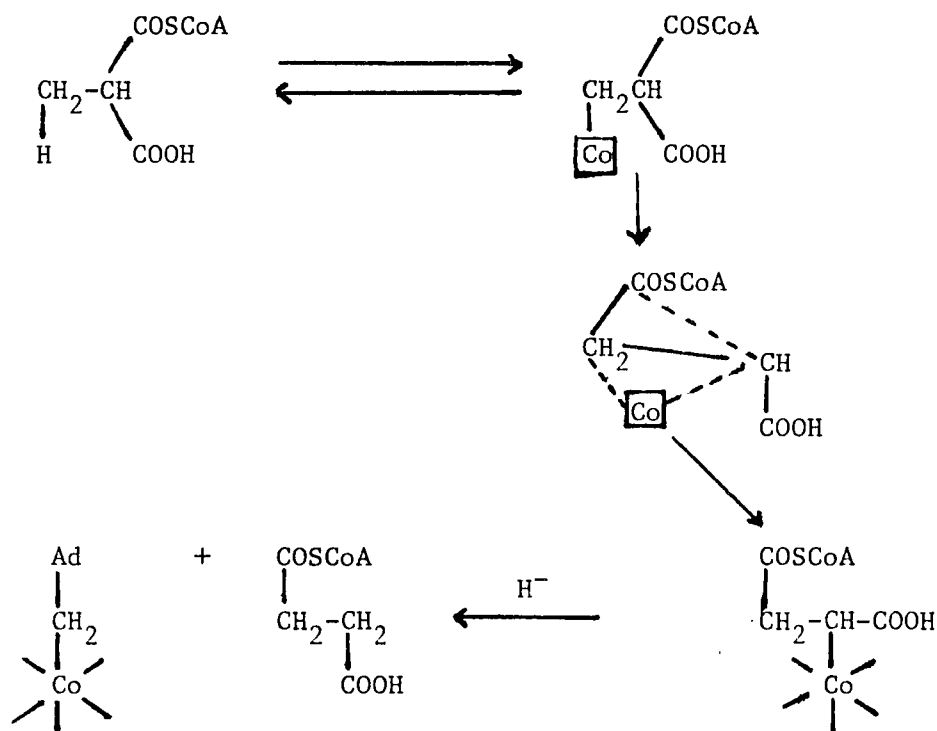
The initial step in the transfer of hydrogen involves the homolytic cleavage of the carbon bond of the coenzyme to form cobalt(II) at the active site and a deoxyadenosyl free radical. The migrating hydrogen is then abstracted from the substrate forming 5'-adenosine and the substrate radical.

Unfortunately, no single mechanism has been universally accepted for the R group migration following the homolysis step. Several years ago, Ingrajam⁸ and coworker proposed a mechanism for the isomerization of methylmalonyl CoA to succinyl CoA which involves ionic cleavage of an organo cobalt intermediate (Scheme II) followed by rearrangement of the resulting carbanion. This rearrangement is comparable to the isomerization mentioned above.

Scheme II

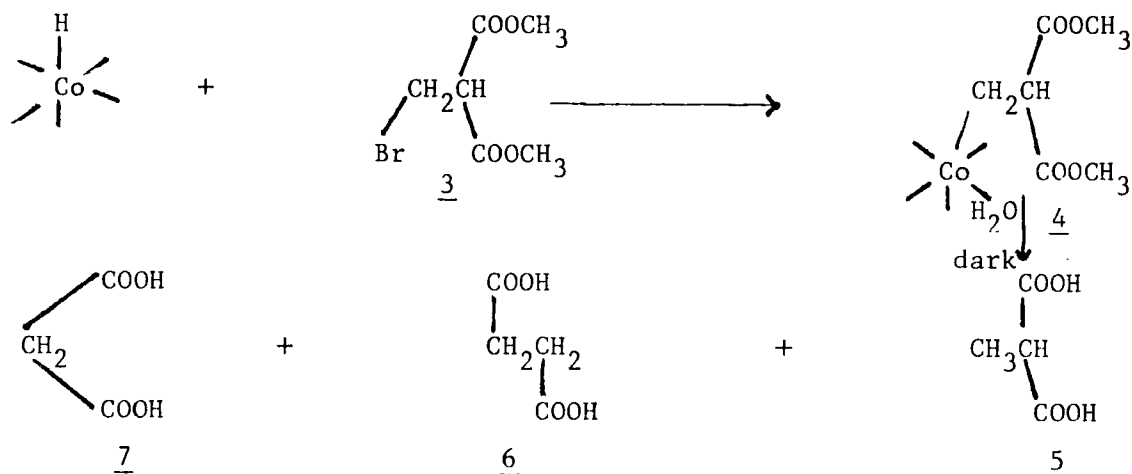


Several possible mechanisms can be written in which a 1,2 shift of the thioester group occurs at a carbonium site with a loss of hydrogen as hydride. Vitamin B₁₂ is required in some of these mechanisms. The mechanism outlined below seems most consistent with existing data.¹⁰⁻¹²



Dowd and Shapiro¹² studied the conversion of L-methylmalonyl CoA to succinyl CoA using bromomethylmalonate as a substrate. The reaction was examined in the absence of the cobamide dependent enzyme, methylmalonyl CoA mutase, found in the microorganism propionibacterium shermanii and in mammalian tissue. By eliminating use of this coenzyme, the specific role of the coenzyme in the transformation could be studied. Reaction of bromomethylmalonate with vitamin B₁₂ gave 4 which models the intermediate in the naturally occurring interconversion. Compound 4 decomposed upon being exposed to light to form hydroxycobalamin. In the dark, the products formed were malonic (7, 18%), methylmalonic (5, 13.6%) and succinic (6, 3.7%) acids as shown in the scheme below.

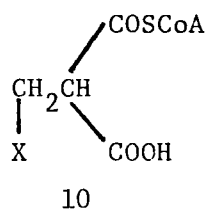
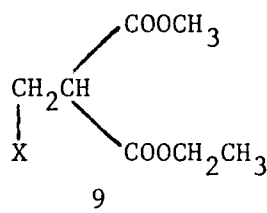
Scheme III



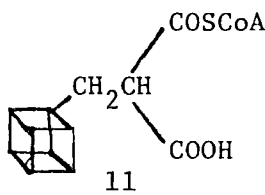
This study gave strong chemical evidence that there is an interaction between the substrate and the cobalt atom of vitamin B₁₂.

Four mechanisms for the reversible isomerization of methylmalonyl CoA to succinyl CoA have been proposed (Table 2). Insufficient data has been presented to establish any one of these as "the" mechanism by which the isomerization takes place.

Prior to the studies done by Dowd and Shapiro, Cole and Johnson¹³ designed compounds 9-11 as substrates to study the enzyme mediated reaction.



X=OH, OTs, Br, NH₂



The models were designed based in part on a report that ethylmalonyl CoA can serve as substrate in the methylmalonyl CoA mutase reaction.¹⁴ The cyclopropyl group is only slightly larger than the ethyl group and would be expected to be a suitable substrate. Additionally, cyclopropyl carbonyl systems are known to undergo rearrangement when an electron deficient atom is generated alpha to the ring. Isolation of 14 from the reaction starting with pure 12 would provide strong evidence in favor of a carbonium ion species as an intermediate.¹⁴ The cubyl substrate also undergoes rearrangement upon generation of

Table 2. Postulated Mechanisms for the Carbon-Skeleton
Rearrangement in Coenzyme B₁₂-Catalyzed Reactions.*

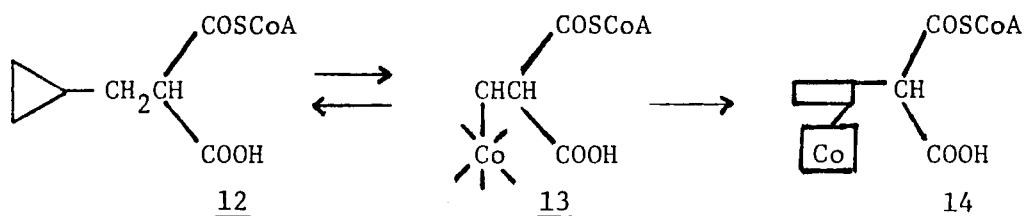
Mechanisms	Enzyme
1. Radical Rearrangement	Diol dehydrase**
2. Carbonium ion Rearrangement	Indefinite
3. Carbanion Rearrangement	Methylmalonyl CoA mutase***
4. Ionic Rearrangement	Diol dehydrase***

*Taken from R. H. Abeles and D. Dolphin, Acc. Chem. Res., 9, 114 (1976).

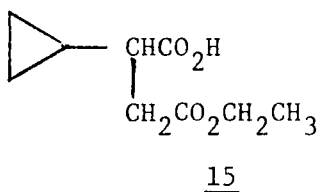
**Taken from J. D. Roberts and R. H. Mazur, ibid., 73, 2509 (1951).

***Taken from B. M. Babior, J. Biol. Chem., 245, 1755 (1970).

an electron deficient atom alpha to the ring. Because the rearrangement is irreversible, identification of products isolated from the enzymatic reaction would provide unequivocal evidence of a carbonium ion formed at some stage in the rearrangement.



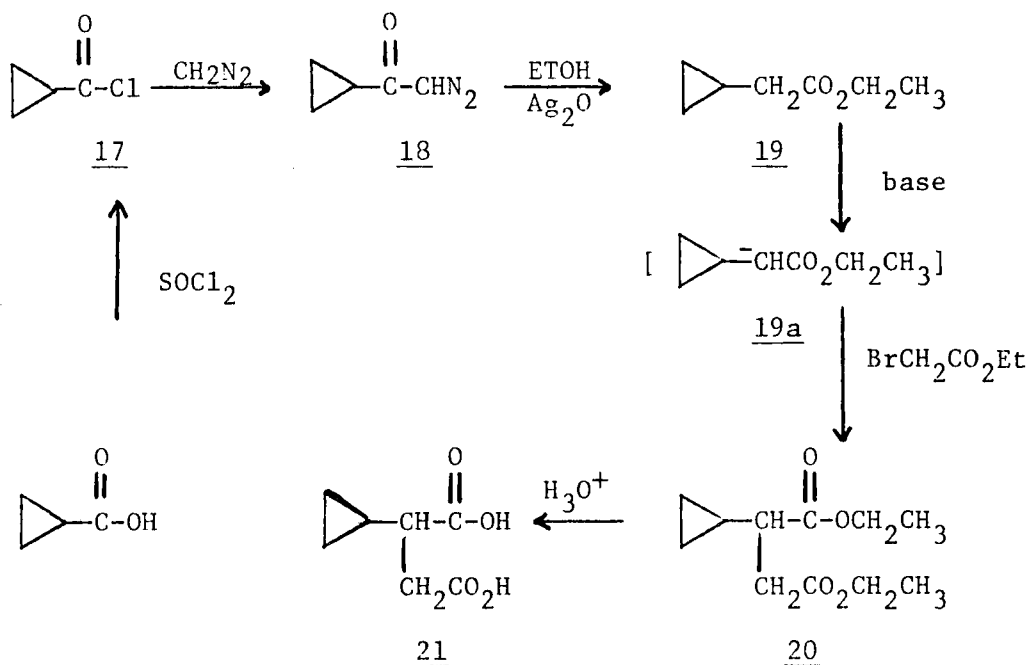
The synthetic approach used by Gilliard¹⁵ for formation of 9, where $\text{X}=\text{OH}$, proved to be unsuccessful. The hydroxyester was too unstable to be useful for studying the methylmalonyl CoA conversion. Because of this instability of methylmalonic acid derivatives, efforts were directed toward the reverse reaction which would be more amenable to chemical synthesis of stable derivatives. Accordingly, the cyclopropyl derivative 20 was designed as a model system for studying the reverse reaction -- conversion of succinyl CoA to methylmalonyl CoA to generate a system that could support or refute the postulation of a cationic intermediate in the isomerization.



RESULTS AND DISCUSSION

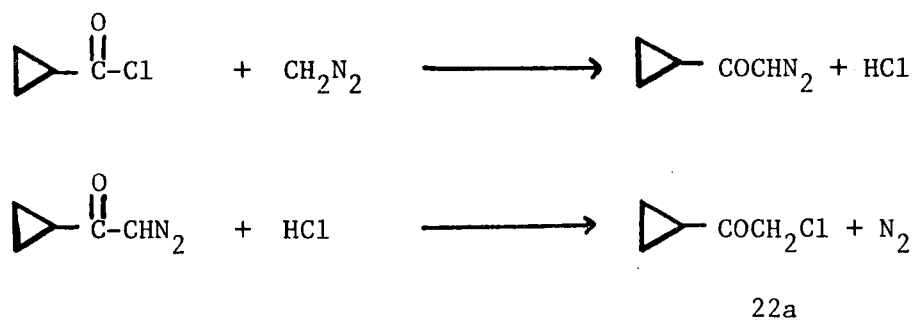
In order to examine the conversion of succinyl CoA to methylmalonyl CoA and to elucidate the structure of any intermediate species, this present study was undertaken to synthesize a suitable model compound. The reaction scheme for preparation of model compound 15 is shown in Scheme IV.

Scheme IV



Ethylcyclopropyl acetate was prepared according to the Arndt-Eistert Synthesis. Reaction of thionyl chloride with cyclopropane carboxylic acid gave 17 in 95 per cent yield. Nuclear magnetic resonance and infrared spectra results were consistent with the structure of the acid chloride 17. Reaction of 17 with diazomethane

overnight gave diazoketone 18. The infrared spectrum showed characteristic stretches of the compound at $\lambda_{\text{max}} = 3000$, 1710, and 1650 cm^{-1} for the C-H, C-N and C=O stretches, respectively. Because of expected instability of diazoketones, no nuclear magnetic resonance spectrum was taken. The next step in the scheme was carried out immediately. Refluxing 18 in the presence of ethanol and 10 per cent aqueous silver nitrate for 18 hours followed by distillation at atmospheric pressure gave 19. Infrared data confirmed the presence of an ester carbonyl at 1739 cm^{-1} , and the disappearance of the carbon nitrogen stretch at 2110 cm^{-1} . The reaction proceeded as expected but there was a very strong unidentifiable singlet in the nuclear magnetic resonance spectrum at $\delta = 4.66$ ppm. This peak was attributed to the presence of a product, 22a, formed by the following pathway:



The formation of 22a was minimized somewhat by reverse addition of diazomethane to the cyclopropane carboxylic acid chloride 17, but still occurred as a competing reaction. Reaction conditions were varied using carbitol (diethylene glycol monoethyl ether) as the solvent. Diazomethane was generated, dried with potassium hydroxide pellets, decanted, then added to 17. Neither change improved the product yield significantly. The reaction sequence was continued

though attempts to remove the product giving rise to the singlet in the nuclear magnetic resonance spectrum were unsuccessful.

The crude ester 19, contaminated with by-products, was alkylated according to Bachmann's¹⁶ procedure with modifications. Sodium ethoxide in excess ethanol and 19 gave 19a which was not isolated. Alkylation was achieved by adding directly bromoethylacetate to the mixture. The diester 20 was isolated and converted to 2-cyclopropyl succinic acid 21 by two hours reflux with aqueous potassium hydroxide. Infrared and nuclear magnetic resonance data indicated the presence of 22, yet the problem of crystallizing the diacid was magnified by impurities encountered in previous reactions.

The reaction sequence was repeated using commercial cyclopropane carboxylic chloride 17. Reaction of the diazoketone 18 with 10 per cent silver nitrate solution yielded a compound with a carbonyl stretch at 1700 cm^{-1} , and an -OH stretch centered at 3600 cm^{-1} , indicating the formation of an acid. Repeated attempts using a variety of reaction conditions gave similar results. Use of commercial silver oxide also gave no appreciable improvement in yield of the ester.

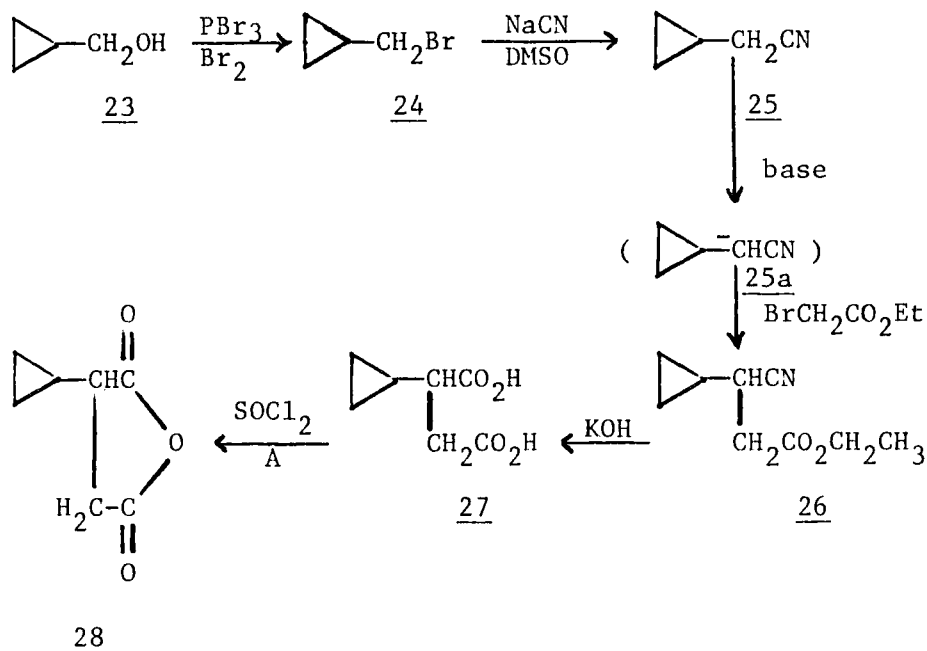
The diazoketone 18 in absolute ethanol was next exposed to ultraviolet radiation. The reaction was monitored by infrared spectroscopy as in previous reactions, until the carbon nitrogen stretch at 2100 cm^{-1} disappeared. An infrared spectrum of the extracted product showed two strong carbonyl stretching vibrations at 1740 cm^{-1} and 1700 cm^{-1} , suggesting the occurrence of a very significant competing reaction.

This mixture was passed through a neutral alumina column using anhydrous ethyl ether as solvent. Different concentrations of ether/carbon tetrachloride solutions were used to maximize separation of components. A 50 per cent by volume mixture of hexane/carbon tetrachloride afforded nearly clean separation. In the first 20 milliliter aliquot the greatest percentage of the desired ester (25 per cent) was obtained. The yield of the ester was too small for this reaction to be considered synthetically useful. The same results were obtained when the reaction was repeated. No analysis was done to determine the structure of the product from the unwanted side reaction.

Various problems were encountered during the synthesis of ethyl cyclopropyl acetate. For example, in certain instances, diazoketone generated rearranged spontaneously such that the carbon-nitrogen stretch at 2100 cm^{-1} disappeared. Comparable amounts of Diazald (N-methyl N-nitroso paratoluene sulfonamide) failed at times to generate sufficient diazomethane to convert all of the acid chloride 17 to the diazoketone. Upon standing, 17 apparently converted to the anhydride, probably as a result of the acid chloride being converted to the acid.

The synthetic route outlined in Scheme I was abandoned following repeated failure to obtain yields of ester 19, in excess of 20 per cent in the last step. Scheme V was then chosen. It was hoped that even though this route is longer than the one previously tried, it would be compensated for by the expected overall increase in yield.

Scheme V



Cyclopropyl carbinyl bromide 24 was initially prepared by the procedure of Wiley¹⁷ with modifications. Yields were increased when phosphorus tribromide was used as the brominating agent.¹⁸ The nitrile 25 was synthesized according to a modified reported procedure.¹⁸ The temperature of the sodium cyanide dimethyl sulfoxide mixture rose sharply upon addition of 24. The product was extracted when the temperature of the mixture reached 50° . It was dried and distilled under atmospheric pressure. When 25 was added to a solution of alcoholic sodium ethoxide in ethyl ether, the solution became turbid. A precipitate formed upon addition of ethylbromoacetate. Nuclear magnetic resonance of the extracted product showed an appreciable amount of unreacted ethylbromoacetate. Attempts

were made to increase the formation of the anion 25a. The nitrile, 25 was refluxed with the base before addition of ethylbromoacetate followed by additional refluxing for 2 hr. Spectroscopic data indicated the presence still of some alkylating agent. Distillation gave no appreciable separation of product from starting material. Gas chromatographic analysis showed retention times of 3.4 and 3.3 minutes, and the distillation was not adjudged a suitable means of purification. Attempts made to separate the product from unwanted starting material by column chromatography were unsuccessful.

Sodium ethoxide proved to be an inadequate base for removal of the active hydrogen. Sodium amide, a much stronger base, was substituted. Addition of sodium amide to a mixture of 25 and ethylbromoacetate in dry toluene produced an exothermic reaction evidenced by gentle refluxing. After addition was complete, the reaction mixture was refluxed for 4 hours. The product was characterized by infrared and nuclear magnetic resonance spectroscopy. Infrared spectrum showed peaks at 2100 cm^{-1} and 1730 cm^{-1} for carbon-nitrogen and a carbonyl stretch, respectively. The nuclear magnetic resonance spectrum seemed consistent with the structure of 26 with some indication of a small amount of unreacted ethylbromoacetate.

Attempts were made to minimize the amount of unreacted starting material. Sodium amide was added to 25 causing immediate refluxing. After 15 minutes the alkylating agent was added slowly. The total mixture was refluxed for four hours. The reaction conditions described in the preceding paragraph produced the best results when the mixture

refluxed overnight. Hydrolysis of 26 obtained from this procedure gave a thick oily substance. The infrared spectrum indicated the formation of an acid with the carbonyl stretch at 1700 cm^{-1} and a broad hydroxyl stretch centered at 3600 cm^{-1} . The diacid 27 was refluxed with thionyl chloride for three hours to give 28 which crystallized out of solution upon standing. Some of the product was lost upon decantation of the thionyl chloride and during the process of washing the crystals with ether. The carbonyl stretch in the infrared spectrum of the remaining product appeared at 1850 cm^{-1} and 1780 cm^{-1} consistent with the presence of anhydride.

EXPERIMENTAL

Infrared (ir) spectra were recorded on a Beckman 4240 or an Acculab 4 spectrophotometer and calibrated against the 6.24 μ band of a polystyrene film. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian Associates A-60 or EM-360 spectrophotometer. Chemical shifts are reported in parts per million downfield from tetramethylsilane.

Removal of solvent in vacuo refers to evaporation at aspirator pressure on a Buchler rotary evaporator.

Cyclopropane Acid Chloride (17).--Cyclopropane carboxylic acid (3.0 g, 34.8 mmol) was placed in a round bottomed flask. To it was added 3.1 g (28.8 mmol) of thionyl chloride. The mixture was refluxed for 45 min and the apparatus then assembled for distillation. The fraction boiling at 119-120° was collected: ir, λ (CCl_4) = 3.33, 5.51 and 7.25 μ ; nmr, δ (CDCl_3) = 2.1 (1 H, multiplet) and 1.2 ppm (4 H, multiplet).

Formation of Cyclopropyl Diazoketone (18).--Diazald (N-Methyl N-nitroso paratoluene sulfonamide, 25 g) mixed with 125 ml of anhydrous ethyl was added slowly to a 150 ml round bottomed flask containing 2.5 g of 17 until the yellow distillate became clear. The resulting mixture was allowed to stand in an ice bath overnight. The ether was removed in vacuo. The recovered material weighed 2.5 g (85.5%): ir, λ (CCl_4) = 3.39, 4.75, and 7.25 μ ; nmr δ (CDCl_3) = 0.6 (multiplet), 2.1 (1 H, doublet), 3.3 (1 H, singlet) and 4.6 ppm (singlet).

Preparation of Ethyl Cyclopropyl Acetate (19).---To a solution of 2.5 g (25.9 mmol) of 18 in 10 ml of absolute ethanol at 55-60° was added 2 ml of aqueous silver nitrate (10%) and 15 ml of ethanol. As soon as the evolution of nitrogen subsided, 5 ml of silver nitrate solution was added. The process was continued until all the silver nitrate solution had been added. The mixture was refluxed until ir spectroscopy indicated the absence of the C=N stretch at $\delta = 4.5$ ppm. Decolorizing carbon was added. The hot solution was filtered and the ethanol removed by distillation at atmospheric pressure to give 2 g (70%) of crude product mixture: ir, λ (CCl_4) = 3.4, 5.75, 8.7 and 9.5 μ ; nmr δ (CDCl_3) = 4.5 (singlet), 4.0 (2 H, quartet), 2.1 (2 H, doublet), 1.0 (3 H, triplet), 0.8 (2 H, multiplet), and 0.5 (4 H, multiplet).

Alkylation of Ethyl Cyclopropyl Acetate (19).---To a solution of sodium ethoxide (1.06 g, 15.5 mmol) in approximately 140 ml of anhydrous ethyl ether contained in a 500 ml Erlenmeyer flask was added 2 g (2.47 mmol) of the ester 19. The flask was stoppered, shaken and allowed to stand. After 5 min, 2.58 g (15.4 mmol) of ethylbromoacetate was slowly added with shaking. The reaction mixture was extracted with ethyl ether and dried over anhydrous sodium sulfate. Removal of the solvent gave a yellow liquid (3 g, crude product, 87.8%): ir, λ (CCl_4) = 3.4, 5.75, 8.7 μ ; nmr δ (CDCl_3) = 4.1 (2 H, quartet), 2.1 (multiplet), 1.0 (multiplet), and 0.5 ppm (4 H, multiplet).

Hydrolysis of Ethyl 2-Cyclopropyl Succinate (21).---Crude diester (20, 2.5 g, 1.17 mmol) was added to a solution of potassium hydroxide in 1.34 ml of water and 5 ml of ethanol. The mixture was refluxed for

3 hr, cooled, extracted with ether, and dried. The weight of recovered dark brown material was 1.13 g (62.11%). Water was added to the crude mixture and ether to induce crystallization. No crystals were afforded after repeated attempts at crystallization: $\text{ir } \lambda (\text{CCl}_4) = 2.6, 3.0, 5.85 \text{ and } 8.25 \mu$.

Preparation of Cyclopropyl Carbinyl Bromide (24) Ph_3P Method.--

Cyclopropyl carbinol (23, 8.09 ml, 100 mmol) was added to 28 g (11.38 mmol) of distilled triphenyl phosphine in 100 ml of dimethyl formamide (previously dried over phosphorus pentoxide) in a nitrogen atmosphere. Bromine (2 ml, 25 mmol) was added until the solution was tinted orange. The reaction temperature was kept below 50° . After the addition was complete the reaction mixture was distilled under reduced pressure. The fraction boiling at $30^\circ/5 \text{ mm}$ was collected. The product was washed with water and dried over anhydrous sodium sulfate. Removal of the solvent gave 10 g (74%): $\text{nmr } \delta (\text{CDCl}_3) = 3.3 (2 \text{ H, doublet}), 0.9 (1 \text{ H, multiplet}), \text{ and } 0.5 (4 \text{ H, multiplet}) \text{ ppm}$.

Preparation of Cyclopropane Carbinyl Bromide.¹⁸--Cyclopropyl carbinol (7.2 g, 100 mmol) was mixed with 30 ml of anhydrous ether and placed in a reaction flask with a magnetic stirrer, drying tube and dropping funnel. Phosphorus tribromide (3.5 ml, 34 mmol) was added slowly after the solution had been cooled to -20° in a Dry Ice/acetone bath. The resulting solution was left stirring overnight. Water (2 ml) was added and the ethereal layer was washed with a saturated solution of sodium bicarbonate. The resulting ether layer was dried (Na_2SO_4), rotary evaporated and distilled giving 10.2 g (75.5%) of the bromide, bp $121^\circ/760 \text{ mm}$.

Preparation of Cyclopropyl Carbinyl Nitrile (25).--Sodium

cyanide (2.6 g, 44 mmol) was partially dissolved in 10.8 ml of dimethyl sulfoxide (dried over calcium hydride). The mixture was contained in a 50 ml reaction flask fitted with thermometer, dropping funnel and magnetic stirrer and was heated to 90°. Cyclopropyl carbinyl bromide (24) was added such that the temperature did not exceed 140°. After addition of the halide, the mixture was stirred until the temperature dropped below 50°, and then extracted with ether. The ethereal layer was washed with a saturated solution of sodium chloride, dried and removed in vacuo. The crude nitrile was distilled (760 mm) and the fraction boiling at 145° was collected giving 2.5 g (84%) of product: $\text{ir } \lambda (\text{CCl}_4) = 4.5, 6.8 \text{ and } 7.0 \mu$; $\text{nmr } (\text{CDCl}_3) = 3.3 (2 \text{ H, doublet}), 1.2 (1 \text{ H, multiplet}) \text{ and } 0.5 \text{ ppm } (4 \text{ H, multiplet})$.

Alkylation of Cyclopropyl Carbinyl Nitrile-Sodium Ethoxide Method.--

To 1.2 g (52.17 mmol) of finely cut sodium metal in a 100 ml reaction flask fitted with a magnetic stirrer, reflux condenser and dropping funnel, was added slowly 39 ml of absolute ethanol. After the addition of the ethanol was complete, the solution was refluxed until all of the sodium had dissolved. To this solution of alcoholic sodium ethoxide in 150 ml of ether was added 4.2 g (52 mmol) of the nitrile 25. The flask was stoppered, shaken and allowed to stand. After 5 min, 5.43 ml (48.9 mmol) of the ethylbromoacetate was added. The reaction mixture was allowed to stand for 10 min. The product was extracted with water. The ethereal layer containing the product was dried and rotary evaporated to give 6 g (69%) of the nitrile. The product was distilled and the fraction boiling at 145° was collected: $\text{ir, } \lambda (\text{CCl}_4) =$

3.8, 4.5, 6.7, 7.3 and 8.5 μ ; nmr δ (CDCl_3) = 4.0 (5 H , multiplet), 3.4 (2 H , quartet), 2.25 (2 H , doublet), 1.1 (multiplet) and 0.5 ppm (4 H , multiplet).

Alkylation of Cyclopropyl Carbinyl Nitrile- NaNH_2 Method.--A

three neck flask containing 4.2 g (51.88 mmol) of the nitrile 25, 8.3 g (49.7 mmol) of ethylbromoacetate and 10 ml of dry toluene was fitted with a dropping funnel, reflux condenser and stopper. To this mixture was added 2.02 g (51.8 mmol) of sodium amide in dried toluene dropwise. The reaction exothermed upon addition of the base. Upon completion of addition of the sodium amide, the mixture was refluxed for 3 hrs to give, after the usual work-up, 4.5 g (51.9%) of material: ir λ (CCl_4) = 2.6, 3.0, 4.6, 5.8, and 8.3 μ ; nmr δ (CDCl_3) = 6.2 (singlet), 4.0 (multiplet), 3.3 (quartet), 2.25 (2 H , doublet), 1.1 (multiplet) and 0.5 (4 H , multiplet).

Alkylation of Cyclopropyl Carbinyl Nitrile (25)- NAH Method.--The nitrile (25, 1.6 g, 18.8 mmol) was refluxed for 2 hrs with sodium hydride in dried benzene. There were no physical changes and ir spectroscopy indicated no reaction had taken place. The reaction mixture was discarded.

Hydrolysis of the Cyanoester (26).--The cyanoester mixture (2 g, 12.61 mmol) was placed in a 50 ml round bottom flask fitted with a magnetic stirrer and reflux condenser. To it was added 30 ml of H_2O and 30 ml of concentrated hydrochloric acid. After 3 hrs, the material was poured into an excess of H_2O and an oily extract was obtained using ethyl ether. No crystallization of the material (1 g, 42.8%) occurred: ir λ (CCl_4) = 2.6, 3.2, 3.5, 7.25, and 8.2 μ .

Formation of Cyclopropyl Succinic Anhydride (28).--Thionyl chloride (7.1 g, 69.6 mmol) was added to a flask containing 1 g (69.22 mmol) of crude diacid 27. The mixture was heated to reflux for 3 hrs and allowed to cool. Crystals were observed after the mixture had been left for 30 min. Some of the thionyl chloride was removed by decantation, the remaining by evaporation, in vacuo. The crystals were washed with ether to give 2 mg of material:
ir λ (CHCl_3) = 3.63, 5.62, 5.85, and 8.26 μ .

CONCLUSION

The first synthetic route for the cyclopropyl model system as outlined seemed very promising initially because of the relatively few steps required to prepare the ethyl ester of cyclopropanecarboxylic acid. Yet this route proved to be most problematic. The sensitivity of the systems involved, i.e., cyclopropane carboxylic acid chloride and the diazoketone, complicated the synthesis and purification. Even though many precautions were taken and much time was devoted to minimizing side reactions, the very prominent unwanted singlet appeared in the nuclear magnetic resonance spectrum of the diazoketone 18, at approximately 4.0 ppm, suggesting a very significant competing reaction.

The set of reactions chosen which ultimately led to the formation of the desired model system contained more steps than the first synthesis attempted. The stability of intermediate products and increased yields gave two distinct advantages over the unsuccessful Scheme I.

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